Photoreceptor Biotechnology

Matthew Hudson
Department of Crop Sciences, University of Illinois, Urbana, Illinois 61801

I. Introduction and Background

Plant photoreceptors influence or control almost all aspects of plant metabolism, growth and development. Only the extent and timing of this control is variable (see other chapters in this volume). Many agriculturally relevant traits are either heavily influenced or completely controlled by photoreceptors. These include seed germination, circadian timing, seedling architecture, bud dormancy, leaf shape and size, stem length and curvature, photosynthetic resource allocation, chloroplast development, chloroplast positioning, flowering time, grain filling and dormancy. In terms of metabolism, the enzymes and other protein components that mediate most of the reactions of photosynthesis are light regulated. Processes such as nitrogen fixation and gas exchange are also regulated by light and influenced by the circadian clock (which is itself is under direct photoreceptor control -see Devlin, this volume). The expression of many other genes and processes are controlled by, or interact with, photoreceptor pathways (see Josse and Halliday, this volume). One agronomically relevant example of this is that defense pathways are strongly influenced by photoreceptor signals (Genoud et al., 2002). Since many photoreceptor-controlled processes are important in determining the yield and suitability of crops, there has been significant interest for many years in using or modifying photoperception for crop improvement.

The control exerted by photoreceptors over so many aspects of plant biology makes them an appealing target for biotechnology approaches. Engineering or smart breeding of photoreceptor genes or their signal transduction components could be used to modify many aspects of plant development and metabolism. There are three major families of plant photoreceptors, the red (R) and far-red (FR) light sensing phytochromes (see Schafer, this volume), the blue / UVA (B) sensing cryptochromes (see Batschauer et al.,

this volume) and the B sensing phototropins (see Christie, this volume). The strategies used to influence desirable traits using these receptors are discussed in Section II.

Of the three families, the phytochromes have so far attracted the most interest for biotechnology applications. This is in part because the phytochromes have been known for longer than other photoreceptors, and constructs to overexpress the genes have been available for some time. Phytochrome overexpressors have been shown to produce strong phenotypes, many of which are desirable from the perspective of yield, harvest time or plant architecture. The blue light (B) sensing cryptochromes influence most of the same processes as the phytochromes. Cryptochrome overexpression thus can also be used to confer desirable phenotypes by overexpression. The phototropins control blue light-induced phototropic responses, chloroplast positioning, leaf expansion and stomatal opening (Kagawa, 2003). While there is potential to modify these responses by altering expression of the phototropins or their signaling partners, there is currently minimal published work on biotechnology applications of the phototropins. Photoreceptor overexpression as a biotechnology tool is discussed in Section III.

Further evidence of the ability of phytochrome photoreceptors to control many aspects of plant growth and development is given by the severe pleiotropic phenotypes of mutants in multiple photoreceptors, or mutants in the synthesis of the phytochrome chromophore (Hudson, 2000). The techniques and challenges of exploiting photoreceptor mutations and natural genetic diversity are discussed in Section IV.

Applications of plant photoreceptors are not limited to the engineering of plant development and metabolism. The unique biochemical properties of plant photoreceptors, particularly the phytochromes, make them attractive candidates for molecular biotechnology with wide-ranging applications. The phytochrome holoprotein is photoconverted between two states by pulses of R and FR. This bistable property of phytochromes could potentially be exploited in a number of ways as a molecular switch. Examples include using phytochrome as a light controlled switch to regulate gene expression, or as a highly fluorescent molecular marker to monitor other biological

processes. Details of these *ex planta* applications are described in Section V of this chapter.

II. Approaches to modification of photomorphogenic responses in crop plants

Dwarfing plants using photoreceptors

The first application for photoreceptor biotechnology became apparent when the first photoreceptor overexpressing plants showed a dwarfed, dark green phenotype (Boylan and Quail., 1989, Keller et al., 1989, Kay et al., 1989). Dwarfing is a widely utilized method of increasing yield or other desirable characteristics by reducing the resources allocated to structural growth. In the case of a dwarf cereal such as wheat or rice, yield is increased by partitioning more photosynthate to the grain at the expense of the structural components of the plant (Salamini, 2003). Dwarfing also renders crops more resistant to mechanical flattening by wind or rain. Dwarf wheat and rice varieties have thus become the choice of most growers.

Dwarf crop varieties in wide use generally carry mutant alleles that affect gibberellin pathways (Peng et al., 1999). However, creation of such mutants in crop species or varieties where they do not yet exist, particularly those with duplicated genomes, is by no means straightforward. Modification of growth regulator pathways using transgenic techniques is a powerful tool, but can be difficult to control. It can lead to pleiotropic dwarfing effects that substantially alter growth and development, making leaves much smaller and hence reducing photosynthetic capacity and potential yield (Curtis et al., 2000). Photoreceptor overexpression is a more controllable tool, with the ability to create dwarfed plants that are not compromised in any aspect of their development that reduces photosynthetic capacity. Such a tool has obvious potential for agronomic application. A recent example of such an approach is the dwarfing of aromatic rice varieties by overexpression of Arabidopsis phyA (Garg et al., 2005). Details of this and other applications of photoreceptor-mediated dwarfing are given in Section III.

The shade-avoidance response

The vast majority of plants have strong competitive morphological and physiological responses to crowding. Vegetation shade is an indicator of the presence of other plants. The responses of plants to vegetation shade are mediated by the perception of light spectral quality, and are collectively termed the "shade avoidance syndrome" (Smith, 1995, Franklin and Whitelam, this volume) The shade avoidance syndrome strongly influences both resource partitioning and growth patterns in almost all plant species investigated, including Arabidopsis, maize, tobacco and some tree species (Smith, 1995, Smith, 1981, 1983, Robson et al., 1996, Gilbert et al., 2001). The syndrome displays common elements in all the species in which it has been identified. Shade-avoiding plants display rapid elongation growth and accelerated reproduction at the expense of leaf expansion and photosynthetic pigment production. The number of embryos that develop on each plant is usually reduced, leading to reduced yield of grain or seed. Shade-avoiding plants also allocate more photosynthate to stem elongation, and less to storage organs such as tubers. Consequently, although shade avoidance is an adaptive response in wild populations, in a densely-grown crop it can lead to yield loss, poor harvest timing and undesirable morphology. Although most modern crops achieve optimal yields when grown at high planting densities, few are bred for responses to light spectral quality. Modification of shade avoidance thus has substantial potential for crop improvement.

Plants distinguish variations in light quality resulting from absorbance of solar irradiation by chlorophyll, even when the total photosynthetically active radiation (PAR) is high. This is possible because the ratio of red to far-red light (R:FR), and hence the equilibrium between the active (Pfr) and inactive (Pr) forms of phytochrome, is strongly proportional to the density of vegetation in the immediate vicinity. (Smith, 1995, Smith and Whitelam, 1997). This proportionality is caused by the depletion of R, with respect to FR, in light transmitted through or reflected from the leaf canopy. Plants are therefore sensitive to crowding to a large extent because they respond to the spectral quality of vegetation shade via the phytochrome family of photoreceptors (see Franklin and Whitelam, this volume, Devlin, this volume, Casal et al., 1997).

Importantly, mutants in the genes encoding light stable phytochromes, particularly phyB, have a pleiotropic phenotype which includes increased elongation, increased apical dominance, reduced chlorophyll levels per unit leaf area and early flowering. This simulates the effect of vegetation shade, probably because both leaf area and flowering time are controlled by Pfr levels in light grown plants. Although phyB plays a dominant role in shade avoidance (Quail 1994) other phytochromes also play a role (Smith and Whitelam, 1997). Just as phyB mutants can simulate an extreme shade-avoidance response, plants that overexpress phytochromes can be used to reduce the extent to which the shade-avoidance syndrome influences plant morphology and development. PhyB overexpression has thus been used successfully to modify shade avoidance characteristics, and achieve increased yield in field crops (see Section III).

The phytochrome A (phyA) photoreceptor has a unique ability to respond more strongly to far-red than to red light, and phyA overexpression (see Section III) has proved to be particularly effective in antagonizing shade avoidance responses (Casal et al., 1997, McCormac et al., 1992). The transcription of the wild type phyA gene is repressed in response to light, and the protein is degraded (See Schafer, this volume). The far-red high irradiance response (FR-HIR) mediated by phyA is therefore normally observed only in etiolated seedlings or de-etiolating seedlings in dense canopies. Overexpression of phyA with a constitutive, viral promoter can extend the FR-HIR into de-etiolated plants, creating an artificial response which is antagonistic to the shade-avoidance syndrome (McCormac et al., 1992). This in turn can be used to increase the harvest index of crops grown in dense stands (Robson et al., 1996).

Control of gene expression and shade avoidance

The challenge of using photoreceptors to modify photomorphogenesis for crop improvement lies in the specific targeting of its many facets. Modification of downstream components of shade-avoidance has the potential to target specific responses within the photoreceptor signaling pathways, and could therefore provide finer tools to control the responses of crop plants to crowding. Knowledge of the mechanisms of shade avoidance could lead to specific targeting of this response without influencing the other characteristics controlled by photoreceptors themselves. For example, it could be possible

to modify resource allocation in response to canopy shade without altering the time of flowering or harvest. Despite the substantial understanding of the role of phytochrome in mediating shade-avoidance responses, however, the molecular events downstream of the perception of R:FR by phytochrome are still incompletely characterized.

Three candidate factors are known in Arabidopsis that could be used to mediate such fine control of the shade-avoidance pathways – PHYTOCHROME INTERACTING FACTOR 3-LIKE 1 (PIL1), ATHB-2 (also known as homeobox-leucine zipper protein 4 (HAT4)) and LONG HYPOCOTYL IN FAR-RED1 (HFR1). The basic helix-loop-helix (bHLH) transcription factor PIL1 was identified as a gene whose mRNA is rapidly induced under low R:FR light conditions. Loss-of-function *pil1* mutants display several phenotypes that indicate PIL1 is necessary for shade-avoidance responses to transient low red:far-red light (Salter et al. 2003). ATHB-2 is a homeodomain-leucine zipper (HD-zip) protein that, like PIL1, is strongly regulated by R:FR (Carabelli et al., 1993, 1996). Overexpression of ATHB-2 causes effects on cell elongation consistent with shade-avoidance, and antisense repression of the gene has an opposite effect (Steindler et al., 1999).

The *hfr1* mutant has a strong effect on the phenotype of shade-avoiding plants. This mutant was isolated because it has a reduced response to FR in etiolated seedlings, implying a role in phytochrome A signaling responses (Fairchild et al. 2000; Fankhauser and Chory 2000; Soh et al. 2000). The *HFR1* transcript, which, like PIL1, encodes a basic helix-loop-helix transcription factor, is strongly and rapidly induced in wild-type plants in response to low R:FR. However, the mutant also shows greatly *increased* shade avoidance responses, implying that HFR1 acts as negative regulator of shade avoidance, perhaps in order to prevent an excessive response causing the death of the seedling (Sessa et al., 2005).

Although ATHB-2, PIL1 and HFR1 are all clearly involved in the mediation of changes in gene expression leading to shade-avoidance, there is more work to be done before the mechanism is fully understood. However, since they do not seem to be involved in global photomorphogenic responses, these loci provide the potential to target shade avoidance responses specifically, using traditional genetic or transgenic techniques as part of crop breeding programs.

Alteration of the timing of flowering

The ability to alter at will the time of year at which a crop flowers or is ready for harvest has obvious potential for crop improvement. By the same means, depending on the organism, it may be possible to control the timing of fruit ripening, tuberization, grain filling and other related traits. Overexpression of phytochromes generally leads to later flowering (Robson and Smith, 1997). Loss-of-function mutants in phytochrome genes, for example the Ma3R allele of sorghum (Childs et al., 1997), tend to flower early and/or be insensitive to photoperiod, generally by flowering earlier under non-inductive conditions (see Section IV). Antisense ablation of phytochrome B transcript removes the photoperiod requirement for tuberization in potato (Jackson et al., 1996). Mutations in the cryptochrome genes also cause reduced sensitivity to photoperiod, but have the converse effect (causing plants to flower later under inductive conditions), in Arabidopsis (Guo et al., 1998, El-Din El-Assal et al., 2003). In contrast, cryptochrome mutants of pea are early flowering (Platten et al., 2005). In addition to the complexity added by the different photoperiodic responses of different species, altered photoreceptor levels affect many other aspects of phenotype and cause pleiotropic effects. This may make photoreceptor modification too blunt an instrument to alter flowering / harvest times of crops without affecting yield.

However, as for shade avoidance, there are other examples of non-photoreceptor signal transduction components, usually transcription factors, which have a significant effect on flowering time. The signaling components controlling floral induction are better characterized, and have been known for longer, than those involved in shade-avoidance. These factors therefore provide a means of engineering the timing of flowering without influencing other aspects of photomorphogenesis. The field is too broad to review in detail here; examples include the zinc-finger protein CONSTANS (CO) (Putterill et al., 1995), the MYB family regulator LATE ELONGATED HYPOCOTYL (LHY) (Schaffer et al., 1998) and the transcription factor INDETERMINATE1 (ID), which is required for the transition to flowering in maize (Colasanti et al., 1998). There are relatively few examples so far of biotechnology being used to alter photoperiodic flowering in plants using these signaling components. Flowering time (hence generation time) has been

modified in citrus trees using overexpression of the transcription factors LEAFY and APETALA1 (AP1) (Pena et al., 2001). As another example, the discovery of the role of VERNALIZATON2 (VRN2) in the control of flowering in wheat (Yan et al, 2004) is an example of a genetic mechanism which leads to a potential future biotechnology application in the control of flowering time. The authors demonstrate that a transgenic RNAi approach can reduce *VRN2* transcript levels and accelerate flowering time of winter wheat by more than one month.

Taxonomic differences and similarities in higher plants

The use of photoreceptor biotechnology to influence patterns of growth and development is complicated by the taxonomic differences in photoreceptors and photomorphogenesis between plant species. The developmental effects of light signals on plants with diverse body plans are necessarily different. In addition, the photoreceptor systems themselves have undergone independent evolution within the angiosperms. The best characterized example of this is the phytochromes (Matthews, 2005). Based on data from rice and other monocot species there are three phytochromes genes, *PHYA*, *PHYB* and *PHYC*, in monocotyledons. This is true except where genome duplication or polyploidy has multiplied the complete family, as is the case in maize (Sawers et al., 1995). In dicotyledons, it is also usual to have one *PHYA* gene and one *PHYC* gene. However, all of the dicots examined so far have multiple B-type phytochromes (in Arabidopsis, designated B, D and E; in other species often designated B1, B2 etc). Complete genome sequencing has now provided firm evidence that there are no more than five phytochrome genes in Arabidopsis, and no more than three in rice. The greater diversity of B-type phytochromes in the dicots may indicate increased selection pressure on these genes, which may in turn reflect divergent evolution of shade-avoidance responses. Whatever the evolutionary implications, this fact complicates the design of experiments intended to modify phytochrome responses by transferring phytochrome genes between species.

In addition to the above differences, the responses mediated by phyA, phyB and phyC vary between monocots and dicots, as indicated by the phenotypes of knockout

mutants. The full sets of five phytochrome mutants in Arabidopsis (Franklin and Whitelam, 2004) and three phytochrome mutants in rice (Takano et al., 2005) are now available. The contrasting roles of the evolutionarily orthologous phytochromes in these species indicate that photosensory function cannot be assumed on the basis of the evolutionary relationships of the photoreceptors.

In Arabidopsis, phyA mediates seeding responses to FR in the HIR response mode, and responses to R and FR in the VLFR response mode. In contrast, phyB mediates most of the responses to R in light-grown plants, and is the predominant receptor for the classic R/FR photoreversible LFR response. Shade-avoidance, or R:FR, is also primarily the role of phyB in wild-type plants although all the phytochromes seem to contribute to this (Smith and Whitelam, 1997, Franklin and Whitelam, 2004). The phenotypes of phyC mutants are more subtle, and overlap somewhat with phyB FR (Franklin et al., 2003; Monte et al., 2003).

In contrast, the perception of R/FR photoreversible responses in rice is mediated by both phyA and phyB. Both phyA and phyC can mediate responses to continuous FR, and phyC does not appear to be involved in the perception of continuous R (Takano et al., 2005). These significant differences highlight the difficulty in applying breeding or transgenic photomorphogenic strategies to crop improvement in different species without a detailed knowledge of the underlying mechanisms of photomorphogenesis in the species under investigation.

Hints of different signaling mechanisms of photomorphogenesis between higher plants have also emerged from transgenic experiments. The strongest dwarfing obtained by phytochrome overexpression has been by using monocot *PHYA* genes from oat or rice, introduced into dicots such as tobacco (Kay et al., 1989, Keller et al., 1989, Robson and Smith, 1997). When extra copies of the native tobacco phyA gene are introduced into tobacco under the control of the same promoter, the phenotype generated is much more subtle (Hudson, 1997). The effect of oat phyA overexpression in rice or wheat is marginal (Clough et al., 1995, Schlumukhov et al., 2003). Conversely, the introduction of an *Arabidopsis PHYA* gene into rice causes significant dwarfing and the promise of substantial increase in yield (Garg et al., 2005). A likely explanation for this observation is that the feedback controls acting post-transcriptionally on the native phytochromes

(especially phyA) are not able to control introduced phytochrome genes as tightly. This can be explained by the introduced coding sequences being from a distantly related species, and thus substantial peptide sequence divergence from the native protein. Consequently, while the use of phytochrome modifications across species boundaries is not well understood, it may prove to be an important tool in the successful use of photomorphogenic modification by transgenic techniques, particularly in cases such as aromatic rice varieties, when the target phenotype is yield increase by dwarfing.

III. Modification of photomorphogenesis using genetic transformation – the state of the art

Plants transgenic for phytochromes

A large number of plant species amenable to transformation have been modified by the introduction of expression cassettes designed to overexpress phytochrome genes. Patents have been filed on the use of phytochrome constructs to cause dwarfing, to modify the shade-avoidance responses or to alter flowering or cropping times (see Section II), and many investigators have applied these techniques to different species and genotypes. The overexpression of phytochrome can lead in many cases to significant dwarfing and to substantial increases in yield (Figure 1). The crop and related model plant species in which phytochrome overexpression has been successfully practiced are given in Table 1 (for lower plant phytochrome expression experiments, see Robson and Smith, 1997). Most is known about the behavior of phytochrome overexpression constructs in tobacco, potato and Arabidopsis than in other species, since genetic transformation of these plants has been straightforward for some time. However, improved transformation technologies have led to phytochrome overexpression being applied in rice (Clough et al., 1995, Garg et al., 2005) and wheat (Schlumukhov et al., 2003).

One of the best examples of the potential of phytochrome expression to increase yield in plants is the overexpression of phytochrome in potato. Additional copies of the potato *PHYA* and Arabidopsis *PHYB* genes have been introduced into potato under the control of the 35S promoter (Heyer et al., 1995, Thiele et al., 1999). Overexpression of *PHYA*

leads to dwarfing and a reduced response to R:FR (Heyer et al., 1995). Overexpression of *PHYB* leads to substantially increased tuberization (Figure 1) and a greater tuber yield from plants grown in controlled environments (Theile et al., 1999). This result extends to field-grown plants, where phyB overexpression causes significantly increased tuber yields in densely-grown plots (Boccalandro et al., 2003). The effect of phyB overexpression in field-grown potato is consistent with a reduced shade-avoidance phenotype (Section II). The source of the increased yield is likely to be altered resource partitioning, and this method may thus provide a general means of increasing the yields of potato tubers, likely without the need for increased use of artificial fertilizers or pesticides.

In addition, a quantitative difference in tuberization in *S. tuberosum ssp. andigena* is observed in phyB antisense transgenics (Jackson et al., 1996). In this potato subspecies, tuberization is normally dependent on short day conditions. The antisense ablation of the *PHYB* transcript allows tuberization to occur under long day conditions, making this an example of the modification of yield timing characteristics using photoreceptor biotechnology (see Section II).

Tobacco is also a good model in which to study the effects of dwarfing and of shade avoidance, since it has a simple, consistent growth habit and puts a significant amount of resources into the formation of internodes. By redirecting the resources devoted to stem elongation into leaf development, there is the potential to achieve a theoretical increase in yield, without complications of altered flowering or harvest time associated with grain crops or tubers. Robson et al (1996) demonstrated that relatively subtle increases in phyA levels (using the *PHYA* gene from *Avena sativa*) can cause dwarfing in tobacco plants which is conditional on the plants being grown in dense stands (proximity-conditional dwarfing). The result of this is an increased harvest index in the transgenic plants, which allocate more resources to leaf formation (tobacco “yield”) than to stem formation when grown densely (Figure 2). However, the phenotype and yield of plants in less dense stands (where light is not limiting and dwarfing is less desirable) is little affected, leading to the promise of a crop that could survive extensive damage more effectively.

Monocotyledon crops (wheat and rice) have also been modified through the use of phytochrome overexpression. Significant alteration of yield or morphology has not yet been accomplished by means of *Avena PHYA* introduced into rice or wheat (Clough et al., 1995, Schlumukhov et al., 2003). However, Arabidopsis *PHYA* overexpression in aromatic rice (*Oryza sativa* L. Pusa Basmati-1) has been demonstrated to create a strong dwarfing phenotype, including reduction in plant height, reduction in internode length and diameter, and an increase in panicle number (Figure 1, Garg et al., 2005). Importantly, the dwarfed plants show a substantial increase in yield, at least under greenhouse conditions. This interesting application of photoreceptor biotechnology is especially promising because of the failure of breeders to incorporate the dwarfing genes that have increased yields in other rice varieties into aromatic rice without losing the distinctive flavor of the grain. Phytochrome overexpression therefore provides a ready means of increasing yield by dwarfing in crops where dwarf genotypes are not available, or where introduction of dwarf traits is too complex, as in aromatic rice.

Table 1: Summary of modifications of phytochrome loci in angiosperms using transgenic techniques, and the traits that were modified**.**
(For a discussion of non-angiosperm transgenic experiments, see Robson and Smith, 1997).

Species (cultivar)	Introduced locus	Trait of interest	Reference
Tobacco (Xanthi)	*Avena PHYA* gene, 35S promoter	Dwarfing, light responses	Viestra et al., 1989
Tobacco (SR-1)	rice *PHYA* gene, 35S promoter	Increased expression of light-regulated genes	Kay et al., 1989

Tobacco (Xanthi)	*Avena PHYA* gene, 35S promoter	Reveresed shade-avoidance (proximity-conditional dwarfing)	McCormac et al., 1993; Robson et al., 1996
Tobacco (Hicks / MM)	*Arabidopsis PHYA*, *PHYB*, *PHYC*, 35S promoter	Flowering time / photoperiodism	Halliday et al., 1997
Tobacco (SR-1)	tobacco *PHYA*, 35S and native promoter	Reveresed shade-avoidance (proximity-conditional dwarfing)	Hudson, 1997.
Tomato (Moneymaker)	*Avena PHYA* gene, 35S promoter	Dwarfing, fruit quality	Boylan et al, 1989
Arabidopsis	*Arabidopsis PHYA*, *PHYB* and *PHYC* genes, 35S promoter	Research into seedling de-etiolation	See Robson and Smith, 1997, Franklin and Whitelam, 2004.
Potato (Desiree)	Arabidopsis *PHYB*, potato *PHYA*, 35S promoter	Increased tuber yield with Arabidopsis phyB (modified resource partitioning)	Heyer et al., 1995, Thiele et al., 1999, Boccalandro et al., 2003.
Potato *ssp. andigena* (photoperiodic)	potato *PHYB* antisense, 35S promoter	Timing of tuberization	Jackson et al., 1996
Wheat (Cadenza)	*Avena PHYA* gene, 35S promoter	Dwarfing, shade-avoidance	Schlumukhov et al., 2003
Rice *Oryza sativa*	*Avena PHYA* gene, Arabidopsis	Dwarfing, yield increase and modified	Clough et al., 1995; Garg et al., 2005

(Gulfmont and Basmati)	*PHYA* gene, 35S promoter	resource partitioning in aromatic rice	

Modification of other photoreceptors

Most work with cyptochromes and phototropins has been focused on loss of function mutants, and in Arabidopsis and rice. This is partly because the most successful dwarfing experiments have been performed with phytochrome genes, and partly because the cryptochrome genes have been available for less time. Blue light photoreceptor transgenic experiments are summarized in Table 2. However, the recent results of cryptochrome overexpression in tomato (Giliberto et al., 2005) may change this pattern. The increases in fruit antioxidant content, with very little negative impact, achieved by Giliberto et al. are likely to generate more interest in the use of cryptochrome overexpression to modify plant development, particularly flowering time and fruit composition.

There has been little work to date on the use of phototropin modification for plant or crop improvement. However, the possibility exists that phototropin pathways could be used to ablate phototropic responses where these are undesirable (for example, in any plants grown under artificial lighting).

Table2: Summary of genetic modification of the blue light photoreceptors, and traits that were successfully modified.

Species	Locus	Trait	Reference
Tobacco	Arabidopsis *CRY1*	Enhanced blue, green and UVA light sensitivity	Lin et al., 1995.
Arabidopsis	*CRY1*	Enhanced blue light sensitivity	Lin et al., 1996
Arabidopsis	*CRY2*	“”	Lin et al., 1998.
Tomato	*CRY2*	Vegetative development,	Giliberto et al., 2005

		flowering time and fruit antioxidant content.	
Physcomitrella patens	*PpCRY1a* and *PpCRY1b*	Side Branches in Protonemata, Differentiation and Growth of Gametophores, Auxin response	Imaizumi et al., 2002

Overexpression of signaling components

Currently, modification of morphology by mutation and overexpression of signaling components has been restricted to experimental analysis of signaling pathways (Quail, this volume). These phenotypes can be restricted to a subset of photomorphogenic responses; for example, NDPK2 knockouts seem to be affected in seedling hook opening but not in hypocotyl elongation (Choi et al., 1999). Since this approach has the potential to specifically target certain aspects of photomorphogenesis, it has great potential for biotechnology application. However, since the phenotypes of signaling component transgenics or mutants are generally much less strong than those of photoreceptor transgenics or mutants, it may be more challenging to achieve a substantially altered yield using this approach.

IV. Modification of photomorphogenesis by utilizing genetic diversity

Natural variation in photomorphogenesis

One of the areas of photomorphogenesis research that has made rapid progress in recent years is the understanding of the role of photoreceptors and photomorphogenic alleles in natural variation and evolution. It has been clearly demonstrated that both reduced and increased phytochrome expression measurably reduce the fitness of plants

competing in canopy environments (Schmitt et al 1995). While this reduced fitness would not necessarily be detrimental to a crop (where all plants are genetically identical and thus canopy competition is eliminated) the result demonstrates that selection pressure will continually act on photomorphogenic systems, both in wild plants and in breeding programs where photomorphogenic behavior is not selected for. Consequently, many crop plants are likely to have photomorphogenic traits sub-optimal for yield. This applies especially to the shade-avoidance syndrome, because of the prevalent selection pressure for canopy competition. Variants within the progeny of a breeder's cross that displayed reduced shade avoidance, grown alongside plants with normal responses, will appear unhealthy (as described by Schmitt et al). Such plants would thus probably not be selected by a breeder, unless they were deliberately targeting shade-avoidance, or yield at increased density, as a trait (see below).

Research in this area is currently focused on the evolution and variation in photomorphogenic systems amongst accessions derived from wild populations. A large number of variable photomorphogenic responses have been described amongst related sub-populations of various species (Maloof et al., 2000). The recent advances in describing the molecular basis of these variable responses has been almost entirely generated by using large numbers of wild-derived accessions of the model plant *Arabidopsis thaliana*. To the surprise of many photobiologists, natural photoreceptor mutants exist and survive within wild populations of *Arabidopsis.* The WS accession of *Arabidopsis* is naturally mutated in the phytochrome D gene (Aukerman et al., 1997) and this example has been enforced by the discovery of a natural phyA variant with greatly reduced FR sensitivity in the Lm-2 accession in a screen of 141 accessions for light response (Maloof et al., 2001). In addition to the Lm-2 variant described by Maloof et al, their screen demonstrates that a great deal of natural variation exists amongst the wild-collected Arabidopsis accessions. Significantly, an association mapping study for flowering time has revealed a novel allele of *CRY2* in Arabidopsis (El-Din El-Assal et al, 2001). This indicates the potential of natural variation in photoreceptor sequences to influence another agronomically significant trait, timing of reproduction / harvest. These and other results have led to greatly increased interest in genomic approaches to

analyzing natural variation in Arabidopsis among evolutionary biologists (Maloof, 2003, Shimizu and Purugganan, 2005).

It has recently been demonstrated using microarray profiling that one of the most variable transcripts in expression level between Arabidopsis accessions is the *PHYB* transcript (Chen et al., 2005). This variability can be explained by the presence of a large degree of sequence diversity in the promoter and intron regions of the *PHYB* locus (the regions mostly responsible for the control of transcription). These results give weight to the notion that genetically controlled variation in photomorphogenesis is a very significant component of evolutionary adaptation of plants to diverse environments.

Photoreceptors and photomorphogenic genes as targets for selection in crops

Given the above results, it is likely that genetic diversity in photomorphogenic pathways lies within the germ plasm collections of many crops, forming an untapped resource for crop yield improvement that does not require chemical applications or transgenes. While it is highly likely (see above) that the photomorphogenic systems of crops are sub-optimal in terms of their photomorphogenic responses, it is also likely that many of the morphological traits of modern crop plants have been selected to increase tolerance to higher planting densities (in particular leaf angle, internode length, tillering and timing of flowering). Given their influence on the morphology and resource partitioning in densely grown crops, photoreceptor genes will in some cases determine yield, particularly at high planting densities where shade-avoidance can be a strong factor in yield determination (Robson, 1996; Robson and Smith, 1997). It is probable, therefore, that breeders have exerted some indirect selection on photomorphogenic traits such as shade avoidance during the breeding of modern crops. This is particularly likely when selection is primarily for increased yield at high planting densities.

For example, in maize, much, if not all, of the significant increases in yield delivered by modern cultivars can be attributed to higher tolerance for crowding (Duvick, 1997) rather than an increase in yield on a per-plant basis. It is understood by maize breeders that photosensitivity can have a negative impact (Salamini, 1985) and so breeders may have altered their selection to compensate for this.

Selection for aberrant photomorphogenic traits in mutagenized, inbred populations, and selection for daylength-insensitive flowering within cereal breeding programs, both lead to the isolation of photoreceptor mutants (see Table 3). This demonstrates the ease with which photomorphogenic variants can be isolated within a population under selection. None of the loci or traits thus isolated has yet been of agronomic benefit; even the lines of crop species isolated within field populations tend to have extreme, pleiotropic phenotypes (Childs et al., 1997, Hanumappa et al., 1999). However, it is likely that selection for more subtle photomorphogenic loci could occur without generating the same pleiotropic phenotypes, and that these loci could be significant in determining desirable traits (Sawers et al., 2005). Although increased yield at higher planting densities can be partly explained by increased tolerance to drought and other stresses (Bruce, 2002), the altered morphology of newer cultivars is also likely to play a role (Fellner et al., 2003). One strategy for increasing crop yields further is to understand and maximize the light signaling systems that allow these cultivars to tolerate to high density planting (Maddonni et al. 2001, Maddonni et al. 2002).

Table 3 Alleles that have been isolated in crop species that directly affect photoreceptor function, and traits modified by the mutations. Note that many more mutants exist in model plants such as Arabidopsis (Hudson, 2000).

Species	Locus	Trait	Reference
Tomato	*phyA, phyB1, phyB2, aurea*	Various effects on photomorphogenesis	Kendrick et al., 1997, Weller et al., 2000
Brassica rapa (rapid cycling)	*ein (phyB)*	Elongated internodes, reduced R response	Devlin et al., 1992, 1997
Cucumber	*lh (phyB)*	Long hypocotyls	Lopez-Juez et al., 1992
Pea	*phyA, phyB*	Various effects on photomorphogenesis	Weller et al., 2001

Rice	*phyA, phyB, phyC*	Various effects on photomorphogenesis	Takano et al., 2005
Maize	*elm1*	Height, internode length, flowering time	Sawers et al., 2002
Sorghum	*Ma3R (phyB)*	Photoperiod insensitivity, elongation	Childs et al, 1997
Barley	*BMDR-1* (*phyB)*	Photoperiod insensitivity, elongation	Hanumappa et al., 1999

Few well-controlled studies of the analysis of photomorphogenic variation between genetically distinct inbred cultivars of a particular crop are available. One exception is the study of the light responses of 30 diverse maize inbred line seedlings, grown under monochromatic red (R), far-red (FR) or blue (B) light of similar irradiance, by Markelz et al. (2003). All these lines had functional photomorphogenic signaling pathways, but displayed over 3-fold variation in phytochrome responses, as measured by mesocotyl length under either red or far-red light. Importantly, the North American cultivars in this study displayed attenuated light responses compared to the semitropical and tropical inbred lines. Thus it is likely that North American breeding practices have indirectly selected for genetic loci that reduce light responsiveness in maize (Markelz et al. 2003).

Despite these attenuated photomorphogenic responses, the characterization of a maize phytochrome mutant (Sawers et al. 2002) and the presence of shade avoidance responses in maize (Maddonni et al. 2002, Smith, 1981, 1983) strongly suggest that light responses not only are operational in adult, light grown maize, but have a significant impact on growth and development. Therefore there is still significant potential for alteration and optimization of these responses in maize.

Additionally, the presumed selection for certain photomorphogenic alleles in highly developed crops such as maize and wheat creates another potential application.

These alleles may at some point be transferable, using genetic transformation or some other method, to crops which have not had the benefit of thousands of years of selective breeding.

V. Photoreceptor biotechnology *ex planta*

Using phytochrome to control gene expression

The discovery of the extremely specific binding of the PIF3 bHLH transcription factor to the Pfr form of Arabidopsis phyB (Ni et al., 1999) opens up a number of possible photoreceptor biotechnology applications. For the first time it is possible to create a complete light-signal transduction system in any organism. In the case of yeast, which has no photoreceptor genes and can complete its lifecycle without light, this allows the creation of a tightly-regulated gene expression system (Figure 3, Shimizu-Sato et al., 2002).

In biotechnology and biomedical research, controllable systems of transcription are ubiquitous tools. Most such systems rely on the addition of a small-molecule regulator to induce or repress the synthesis of an mRNA; however once the regulator is added, it cannot easily be removed from the culture, leaving the mRNA synthesis permanently switched "on". Sato and coworkers were able to create a system where expression of a target gene (in their case the LacZ reporter) in yeast can be switched on by a pulse of red light, and switched off again by a pulse of far-red light. The induction of the lacZ reporter in response to a red light pulse was three orders of magnitude within three hours, and this induction was completely prevented by a far-red pulse given after the red pulse. This light-regulated system was created by fusing PIF3 to the transcriptional-activation domain of GAL4 (Gal4AD), and creating a chimeric, chromophorylated and photoreversible phyB-GAL4 DNA binding domain protein (Figure 3A). This creates a system where transcription is controlled by the recruitment of the PIF3:Gal4AD fusion, which in turn is controlled by the photoreversible conformation of phytochrome (Figure 3B). The induction of gene expression can be induced at any time with a pulse of red light, and reversed at any time using a pulse of far-red light (Figure 3C).

Currently, the techniques required to extract and handle the phycocyanobilin chromophore, and culture yeast in darkness with defined light sources, are not easily accomplished outside a plant photoreceptor laboratory. In addition, the chromophore containing media causes some signs of photodynamic toxicity in prolonged illumination (Shimizu-Sato et al., 2002). However, engineering of a chromophore biosynthetic pathway into yeast should be relatively straightforward, since the pathway has already been successfully introduced in bacteria (Gambetta and Lagarias, 2001). Such a chromophore-producing yeast strain would make this approach feasible in most molecular biology laboratories.

There is no fundamental barrier to prevent this technology from being extended to other organisms, for example *Drosophila* embryos or mammalian cell lines. If the idea of systems biology is to be taken seriously, the ability to turn on and turn off expression of genes very rapidly will become indispensable in order to model the dynamics of cellular biochemistry and regulatory networks (Kærn et al., 2003). With chromophore biosynthetic genes added to the system, this method could therefore become a very widely used research tool.

Phytochromes as fluorescent probes

Fluorescent proteins are widely used as fluors in cell biology, microscopy, and in techniques such as RNA detection on "gene chip" microarrays (Zhang et al., 2002). The use of fluorescent protein probes is extremely common in molecular and biochemical research and is now becoming important in medicine also, making the search for more intense fluorescent proteins at new wavelengths increasingly important. Biliproteins such as phycoerythrin have an advantage in many applications over fluorescent dyes because of their high fluorescence quantum yield, and hence high signal: noise ratio.

Although phytochromes are biliproteins like the phycoerythrins, they are not normally fluorescent proteins (fluorescence quantum yield is less than 10^{-3} at room temperature (Brock et al., 1987)). Instead of photons captured by the bilin chromophore being re-emitted as energy at other wavelengths, the energy is used in the photoconversion process and stored in the conformation of the Pfr form, which slowly decays back to the Pr form in darkness. However, when the native chromophore is replaced by phycoerythrobilin (an analog of the natural chromophore which lacks the C15 double bond), the result is an intensely fluorescent, photostable protein that is presumably unable to undergo the Pr – Pfr conversion (Murphy and Lagarias., 1997). The phytofluors have emission maxima in the 580-590nm range, where no fluorescent probes are currently available. They have quantum yields of 0.7 – 0.82, putting them in the same useful range as most of the other widely used fluorescent protein probes. Both of these parameters could likely be altered or improved by judicious site-directed mutation of the

phytofluor apoprotein. The phytofluors may consequently be the first commercial application of phytochrome biotechnology (Fischer and Lagarias, 2004).

Other potential uses of photoreceptors

The unique physical properties of phytochromes have led to a number of other suggestions for their possible utility outside the plant modification. The red-far red photoreversible property of the protein could potentially be used as a method for storing solar energy. It could also form the basis of an optical storage device such as those that have been envisioned for optical computers (Ni et al., 1999). Understanding of the molecular structure of phytochrome is finally becoming more advanced, with a huge advance in the form of a phytochrome crystal structure (Wagner et al., 2005). Knowledge of the physics of the photoconversion process, derived from the structural biology of phytochrome, may aid in the design of light-driven nanomachines, even if phytochrome itself does not play a role in these devices.

VI. Future directions in photoreceptor biotechnology

Genomics is likely to generate new tools for the modification of photomorphogenesis in plants. As the details of photomorphogenesis become clear in more plant species, and the genome projects of crops such as maize draw closer to completion, interest is likely to increase in using photoreceptors or their signaling pathways to cause targeted dwarfing, alter shade avoidance or influence other traits. While the use of genetic transformation is likely to remain important in research into plant photomorphogenesis, knowledge of polymorphisms between crop cultivars will increase as a result of resequencing strategies applied across large germ plasm collections, and will lead to the production of large databases of genetic diversity at the molecular level. Combination of such databases with quantitative trait data is likely to lead to the discovery of photomorphogenic alleles that have arisen during the evolution or breeding of modern crop plants. Given the evidence for the importance of photomorphogenesis in determining yields, such alleles are likely to become the focus of targeted “smart breeding” approaches in order to incorporate complete optimal photomorphogenic systems into elite lines. For these reasons photoreceptors and

photomorphogenesis are likely to become more important in the eyes of crop breeders and physiologists.

In terms of other applications of photoreceptor biotechnology, knowledge of the structure of phytochrome (Wagner et al., 2005) is likely to have significant impacts on the molecular biotechnology uses of phytochrome. It may now be possible to optimize light-driven gene control systems or phytofluors by intelligent domain-swap or site-directed mutagenesis of key residues involved in photoconversion, PIF3 binding or chromophore binding. The ability to make such intelligent, evidence-driven modifications may allow photoreceptor scientists to move out of the "dark ages" and into a new era of advanced protein design.

Acknowledgements

Thanks are due to Dr. Thomas Brutnell for helpful discussions, and to Dr. Karen Kaczorowski and Dr. Kankshita Swaminathan for critical reading of the manuscript. Research in the Hudson laboratory is funded by the United States Department of Agriculture, the Illinois Council on Food and Agricultural Research, the National Center for Supercomputer Applications and the University of Illinois.

References

Aukerman MJ, Hirschfeld M, Wester L, Weaver M, Clack T, Amasino RM, Sharrock RA (1997) A deletion in the PHYD gene of the Arabidopsis Wassilewskija ecotype defines a role for phytochrome D in red/far-red light sensing. Plant Cell 9: 1317-1326

Boccalandro, H.E., Ploschuk, E.L., Yanovsky, M. Y., Sanchez, R. A., Gatz, C. and Casal., J.J. (2003). Increased Phytochrome B Alleviates Density Effects on Tuber Yield of Field Potato Crops. Plant Physiol. 133, 1539-1546.

Boylan, M.T. and Quail, P.H. (1989) Oat phytochrome is biologically active in transgenic tomatoes. Plant Cell, 1, 765-773

Brock H., Ruzsicska BP, Arai T., Schlamann W., et al (1987). Fluorescence lifetimes and relative quantum yields of 124-kilodalton oat phytochrome in H2O and D2O solutions. Biochemistry 26:1412-1417.

Bruce WB, Edmeades GO, Barker TC (2002). Molecular and physiological approaches to maize improvement for drought tolerance. J.Exp.Bot. 53, 13-25.

Carabelli M, Morelli G, Whitelam G, Ruberti I (1996). Twilight-zone and canopy shade induction of the Athb-2 homeobox gene in green plants. Proc Natl Acad Sci U S A. 93, 3530-5.

Carabelli M, Sessa G, Baima S, Morelli G, Ruberti I (1993). The Arabidopsis Athb-2 and -4 genes are strongly induced by far-red-rich light. Plant J. 4, 469-79.

Casal, J.J., Sanchez, R.A., and Yanovsky, M.J. (1997) . The function of phytochrome A. Plant Cell Env. 20: 813-819.

Chen, WJ, Chang SH, Hudson M, Kwan WK, Li J, Estes B, Knoll D, Shi L and Zhu T (2005). Contribution of transcriptional regulation to natural variations in Arabidopsis. Genome Biology 6:R32.

Childs KL, Miller FR, Cordonnier-Pratt M-M, Pratt LH, Morgan PW, Mullet JE (1997). The sorghum photoperiod sensitivity gene, Ma3, encodes a phytochrome B. Plant Physiol. 113: 611–619

Choi, G., Yi, H., Lee, J., Kwon, Y.K., Soh, M.S., Shin, B., Luka, Z., Hahn, T.R., and Song, P.S. 1999. Phytochrome signaling is mediated through nucleoside diphosphate kinase 2. Nature 401: 610-613

Clough RC, Casal JJ, Jordan ET, Christou P and Vierstra RD (1995). Expression of a functional oat phytochrome A in transgenic rice. Plant Physiol 109:1039–1045

Colasanti J, Yuan Z, Sundaresan V. (1998). The indeterminate gene encodes a zinc finger protein and regulates a leaf-generated signal required for the transition to flowering in maize. Cell 93, 491-494.

Curtis IS, Ward DA, Thomas SG, Phillips AL, Davey MR, Power JB, Lowe KC, Croker SJ, Lewis MJ, Magness SL, Hedden P (2000) Induction of dwarfism in transgenic Solanum dulcamara by over-expression of a gibberellin 20-oxidase cDNA from pumpkin. Plant J 23: 329–338.

Devlin, P.F., Rood, S.B., Somers, D.E., Quail, P.H. and Whitelam, G.C. (1992). Photophysiology of the elongated internode (ein) mutant of Brassica rapa: ein mutant lacks a detectable phytochrome B-like polypeptide. Plant Physiol 100: 1442–1447.

Devlin. P.F., Somers, D.E., Quail, P.H. and Whitelam, G.C. (1997). The Brassica rapa elongated internode (EIN) gene encodes phytochrome B. Plant Molecular Biology 34: 37–547, 1997.

Duvick DN (1997). What is yield? p. 332-335 In G. O. Edmeades, B. Banziger, H. R. Mickelson and C. B. Peña-Valdivia (ed.) Developing Drought and Low N-Tolerant Maize. Proceedings of a Symposium, March 25-29, 1996, CIMMYT, El Batan, Mexico. CIMMYT, México, D.F.

El-Din El-Assal, S., Alonso-Blanco, C., Peeters, A.J.M., Raz, V. and Koornneef, M. (2001). A QTL for flowering time in Arabidopsis reveals a novel allele of CRY2. Nat. Genetics 29: 435 - 440

El-Din El-Assal, S., Alonso-Blanco, C., Peeters, A.J.M., Wagemaker, C., Weller, J.L. and Koornneef, M. (2003). The Role of Cryptochrome 2 in Flowering in Arabidopsis. Plant Physiol 133: 1504-1516

Fairchild CD, Schumaker MA, Quail PH (2000). HFR1 encodes an atypical bHLH protein that acts in phytochrome A signal transduction. Genes Dev 14, 2377–2391

Fankhauser C, Casal JJ (2004). Phenotypic characterization of a photomorphogenic mutant. Plant J. 39, 747–760

Fellner M, Horton LA, Cocke AE, Stephens NR, Ford ED, Van Volkenburgh, E (2003). Light interacts with auxin during leaf elongation and leaf angle development in young corn seedlings. Planta 216, 366-376.

Fischer, A.J., Lagarias, J.C. (2004). Harnessing phytochrome's glowing potential. Proc. Natl. Acad. Sci. USA, 101: 17334-1733

Franklin KA, Davis SJ, Stoddart WM, Vierstra RD and Whitelam GC (2003) Mutant analyses define multiple roles for phytochrome C in Arabidopsis photomorphogenesis. Plant Cell 15:1981–1989

Franklin, K.A. and Whitelam, G.C. (2004). Light signals, phytochromes and cross-talk with other environmental cues. J. Exp. Bot. 55, 271-276.

Gambetta, G.A. and Lagarias, J.C. (2001). Genetic engineering of chromophore biosynthesis in bacteria. Proc. Natl. Acad. Sci. 98, 10566-10571.

Garg AK, Sawers RJ, Wang H, Kim JK, Walker JM, Brutnell TP, Parthasarathy MV, Vierstra RD, Wu RJ. (2005). Light-regulated overexpression of an Arabidopsis phytochrome A gene in rice alters plant architecture and increases grain yield. Planta 2005 Sep 1;:1-10 (epub ahead of print).

Genoud, T., Buchala, A.J., Chua, N.-H. and Métraux, J.-P. (2002). Phytochrome signalling modulates the SA-perceptive pathway in Arabidopsis. Plant J. 31: 87-96

Gilbert IR, Jarvis PG, Smith H. (2001). Proximity signal and shade avoidance differences between early and late successional trees. Nature 411, 792-795.

Giliberto L, Perrotta G, Pallara P, Weller JL, Fraser PD, Bramley PM, Fiore A, TavazzaM, Giuliano G. (2005). Manipulation of the blue light photoreceptor cryptochrome 2 in tomato affects vegetative development, flowering time, and fruit antioxidant content. Plant Physiol. 137: 199-208.

Guo H, Yang H, Mockler TC, Lin C (1998) Regulation of flowering time by Arabidopsis photoreceptors. Science 279: 1360–1363

Halliday KJ, Thomas B and Whitelam GC (1997) Expression of heterologous phytochrome A, B or C in transgenic tobacco plants alters vegetative development and flowering time. Plant J 12:1079–1090

Hanumappa, M., Pratt, L.H., Cordonnier-Pratt, M.-M., and Deitzer, G.F. (1999) A photoperiod-insensitive barley line contains a light-labile phytochrome B. Plant Physiol. 119, 1033–1040

Heyer AG, Mozley D, Landschutze V, Thomas B, Gatz C. (1995). Function of phytochrome A in potato plants as revealed through the study of transgenic plants. Plant Physiol. 109: 53-61

Hudson, M. (2000). The genetics of phytochrome signalling in Arabidopsis. Semin. Cell Dev. Biol. 11, 475-583.

Hudson, M.E. (1997). Analysis of phytochrome function in the genus Nicotiana using mutant and transgenic plants. PhD. thesis, University of Leicester, UK.

Imaizumi, T., Kadota, A., Hasebe, M. and Wada, M. (2002). Cryptochrome Light Signals Control Development to Suppress Auxin Sensitivity in the Moss Physcomitrella patens. Plant Cell, Vol. 14, 373-386,

Jackson SD, Heyer A, Dietze J, Prat S. Phytochrome B mediates the photoperiodic control of tuber formation in potato. Plant J. 1996;9:159–166

Kærn, M., Blake, W.J. and Collins, J.J. (2003). THE ENGINEERING OF GENE REGULATORY NETWORKS. Annual Review of Biomedical Engineering 5: 179-206

Kagawa, T. (2003). The phototropin family as photoreceptors for blue light-induced chloroplast relocation. J. Plant Res. 116: 75 - 80

Kay SA, Nagatani A, Keith B, Deak M, Furuya M and Chua N-H (1989) Rice phytochrome is biologically active in transgenic tobacco. Plant Cell 1:775–782

Keller, J. M., Shanklin, J.,Vierstra, R.D., Hershey, H. P. (1989). Expression of a functional monocotyledonous phytochrome in transgenic tobacco. EMBO J. 1989 Apr;8(4):1005–1012.

Kendrick, R.E., Kerckhoffs, H.J., Van Tuinen, A. and Koornneef, M. (1997). Photomorphogenic mutants of tomato. Plant Cell Env. 20: 746-751.

Lin C, Ahmad M, Cashmore AR. (1996). Arabidopsis cryptochrome 1 is a soluble protein mediating blue light-dependent regulation of plant growth and development. Plant J 10: 893-902

Lin, C., Ahmad, M., Gordon, D. and Cashmore, A.R. (1995). Expression of an Arabidopsis Cryptochrome Gene in Transgenic Tobacco Results in Hypersensitivity to Blue, UV-A, and Green Light. Proc Natl. Acad. Sci. USA 92, 8423-8427

Lin, C., Yang, H. Guo, H., Mockler, T., Chen, J. and Cashmore, A. R. (1998) Enhancement of blue-light sensitivity of Arabidopsis seedlings by a blue light receptor cryptochrome 2. Proc Natl. Acad. Sci. USA 95: 2686-2690

Lopez-Juez E, Nagatani A, Tomizawa K-I, Deak M, Kern R, Kendrick R.E. and Furuya M (1992) The cucumber long hypocotyl mutant lacks a light-stable PHYB-like phytochrome. Plant Cell 4: 241–251.

Maddonni GA, Otegui ME Cirilo AG (2001). Plant population density, row spacing and hybrid effects on maize architecture and light attenuation. Field Crop Res 71, 183 193

Maddonni GA, Otegui ME, Andrieu B, Chelle M and Casal, JJ (2002). Maize Leaves Turn Away from Neighbors. Plant Physiol. 130, 1181–89

Maloof JN, Borevitz JO, Weigel D, Chory J. (2000). Natural variation in phytochrome signaling. Semin Cell Dev Biol. 11: 523-30.

Maloof JN. (2003). Genomic approaches to analyzing natural variation in Arabidopsis thaliana. Curr Opin Genet Dev. 13: 576-82.

Maloof, JN, Borevitz, JO, Dabi, T, Lutes, J, Nehring, RB, Redfern, JL, Trainer, GT, Wilson, JM, Asami, T, Berry, CC, Weigel, D, Chory, J (2001) Natural variation of light sensitivity in Arabidopsis. Nature Genet. 29, 441-446.

Markelz NH, Costich DE, Brutnell, TP. (2003) Photomorphogenic Responses in Maize Seedling Development. Plant Phys 133, 1578-91

Mathews S. (2005). Phytochrome evolution in green and nongreen plants. J Hered. 96: 197-204.

McCormac, A.C., Wagner, D., Boylan, M.T., Quail, P.H., Smith, H., and Whitelam, G.C. (1993). Photoresponses of transgenic Arabidopsis seedlings expressing introduced phytochrome B-encoding cDNAs: Evidence that phytochrome A and phytochrome B have distinct photoregulatory functions. Plant J. 4, 19–27

McCormac, A.C., Whitelam, G.C., and Smith, H. (1992). Light-grown plants of transgenic tobacco expressing an introduced oat phytochrome A gene under the control of a constitutive viral promoter exhibit persistent growth inhibition by far-red light. Planta 188: 173−181.

Monte, E., Alonso, J. M., Ecker, J. R., Zhang, Y., Li, X., Young, J., Austin-Phillips, S. and Quail, P. H. (2003). Isolation and Characterization of phyC Mutants in Arabidopsis Reveals Complex Crosstalk between Phytochrome Signaling Pathways. Plant Cell 15, 1962-1980.

Morelli G, Ruberti I (2002). Light and shade in the photocontrol of Arabidopsis growth. Trends Plant Sci 7, 399-404

Murphy, J.T., and Lagarias, J.C. (1997) The phytofluors: A new class of fluorescent protein probes. Curr. Biol. 7:870-876

Ni M, Tepperman JM, Quail PH (1999). Binding of phytochrome B to its nuclear signalling partner PIF3 is reversibly induced by light. Nature 400, 781–784

Peña, L., Martín-Trillo, M., Juárez, J., Pina, J.A., Navarro, L. and Martínez-Zapater, J.M. (2001). Constitutive expression of Arabidopsis LEAFY or APETALA1 genes in citrus reduces their generation time. Nature Biotech. 19, 263 – 267.

Peng, J., Richards, D.E., Hartley, N.M., et al (1999). 'Green revolution' genes encode mutant giberellin response modulators. Nature 400: 256-261.

Platten JD, Foo E, Elliott RC, Hecht V, Reid JB, Weller JL.(2005). Cryptochrome 1 contributes to blue-light sensing in pea. Plant Physiol. 139:1472-82.

Putterill J, Robson F, Lee K, Simon R, Coupland G. (1995). The CONSTANS gene of Arabidopsis promotes flowering and encodes a protein showing similarities to zinc finger transcription factors. Cell 80, 847-57.

Quail, P.H. (1994). Phytochrome Genes and their Expression. In: Kendrick, R.E. and Kronenberg, G.H.M., eds., Photomorphogenesis in plants. Dordrecht: Kluwer. 2nd ed. pp 71-104

Robson PR, McCormac AC, Irvine AS, Smith H. (1996) Genetic engineering of harvest index in tobacco through overexpression of a phytochrome gene. Nat Biotechnol. 14, 995-998.

Robson, P.R.H. and Smith, H. (1997). Fundamental and biotechnological applications of phytochrome transgenes. Plant Cell Env. 20: 831-839.

Salamini F (1985) Photosensitivity in maize: evaluation, genetics, and breeding for insensitivity. In A Bandolini, F Salamini, eds, Breeding Strategies for Maize Production Improvement in the Tropics. Food and Agriculture Organization of the United Nations and Istituto Agronomico per L'Oltremare, Florence, Italy, pp 143-157

Salamini, F, (2003). Hormones and the Green Revolution. Science 302. 71 – 72

Salter MG, Franklin KA, Whitelam GC. (2003). Gating of the rapid shade-avoidance response by the circadian clock in plants. Nature 426, 680-683.

Sawers JHR, Linley PJ, Farmer PR, Hanley NP, Costich DE, Terry MJ and Brutnell TP (2002). elongated mesocotyl1, a Phytochrome-Deficient Mutant of Maize. Plant Physiol. 2002, 155-163.

Sawers RJ, Linley PJ, Gutierrez-Marcos JF, Delli-Bovi T, Farmer PR, Kohchi T, Terry MJ, Brutnell TP (2004) The Elm1 (ZmHy2) Gene of Maize Encodes a Phytochromobilin Synthase. Plant Physiol 136, 2771-81.

Sawers, R.J.H., Sheehan, M.J. and Brutnell, T.P. (2005). Cereal phytochromes: targets of selection, targets for manipulation? Trends Plant Sci. 10:138-143.

Schaffer R, Ramsay N, Samach A, Corden S, Putterill J, Carre IA, Coupland G. (1998). The late elongated hypocotyl mutation of Arabidopsis disrupts circadian rhythms and the photoperiodic control of flowering. Cell 93, 1219-29.

Schmitt, J., McCormac., A.C. and Smith, H. (1995). A test of the adaptive plasticity hypothesis using transgenic and mutant plants disabled in phytochrome-mediated elongation responses to neighbors. Am. Nat. 146: 937-953.

Sessa, G., Carabelli, M., Sassi, M., Ciolfi, A., Possenti, M., Mittempergher, F., Becker, J., Morelli, G and Ruberti, I. (2005). A dynamic balance between gene activation and repression regulates the shade avoidance response in Arabidopsis. Genes Dev. 19:2811-2815.

Shimizu-Sato, S., Huq, E., Tepperman, J.M., and Quail, P.H. (2002). A light-switchable gene promoter system. Nat. Biotechnol. 10, 1041–1044

Shlumukov LR, Barro F, Barcelo P, Lazzeri P, Smith H (2001) Establishment of far-red high irradiance responses in wheat through transgenic expression of an oat phytochrome A gene. Plant Cell Environ 24: 703-712

Shmizu, K.K. and Purugganan, M. D. (2005). Evolutionary and Ecological Genomics of Arabidopsis. Plant Physiol. 138: 578-284.

Smith H, Whitelam GC (1997). The shade avoidance syndrome: multiple responses mediated by multiple phytochromes. Plant Cell Environ 20: 840-844

Smith H. (1981). Evidence that Pfr is not the active form of phytochrome in light-grown maize. Nature 293, 161-165.

Smith H. (1983). Is Pfr the active form of phytochrome? Phil. Trans. Royal Soc. London, 303, 443-452.

Smith, H. (1995) Physiological and ecological function within the phytochrome family. Annu. Rev. Plant Physiol. Plant Mol. Biol., 46, 289-315

Soh, M.-S., Kim, Y.-M., Han, S.-J., and Song, P.-S. 2000. REP1, a basic helix–loop helix protein, is required for a branch pathway of phytochrome A signaling in Arabidopsis. Plant Cell 12: 2061–2073

Steindler C, Matteucci A, Sessa G, Weimar T, Ohgishi M, Aoyama T, Morelli G, Ruberti I. (1999) Shade avoidance responses are mediated by the ATHB-2 HD-zip protein, a negative regulator of gene expression. Development. 126:4235-45.

Takano M, Inagaki N, Xie X, Yuzurihara N, Hihara F, Ishizuka T, Yano M, Nishimura M, Miyao A, Hirochika H, Shinomura T. (2005). Distinct and cooperative functions of phytochromes A, B, and C in the control of deetiolation and flowering in rice. Plant Cell. 17:3311-25.

Thiele A, Herold M, Lenk I, Quail PH, Gatz C. (1999) Heterologous expression of Arabidopsis phytochrome B in transgenic potato influences photosynthetic performance and tuber development. Plant Physiol. 120: 73-82.

Wagner JR, Brunzelle JS, Forest KT, Vierstra RD. (2005). A light-sensing knot revealed by the structure of the chromophore-binding domain of phytochrome. Nature. 438: 325-331.

Weller JL, Beauchamp N, Kerckhoffs LHJ, Platten JD, Reid JB (2001) Interaction of phytochrome A and B in the control of de-etiolation and flowering in pea. Plant J 26: 283–294

Weller JL, Schreuder MEL, Koornneef M, Kendrick RE (2000) Physiological interactions of phytochromes A, B1 and B2 in the control of development in tomato. Plant J 24: 345–356

Yan, L. Loukoianov, A, Blechl, A. et al. (2004) The Wheat VRN2 Gene Is a Flowering Repressor Down-Regulated by Vernalization. Science 303. 1640 – 1644

Zhang, J., Campbell, R.E., Ting, A. Y. and Tsien, R. Y. (2002). Creating new fluorescent probes for cell biology. Nat. Rev. Mol. Cell. Biol. 3: 906-918.

Figure Legends

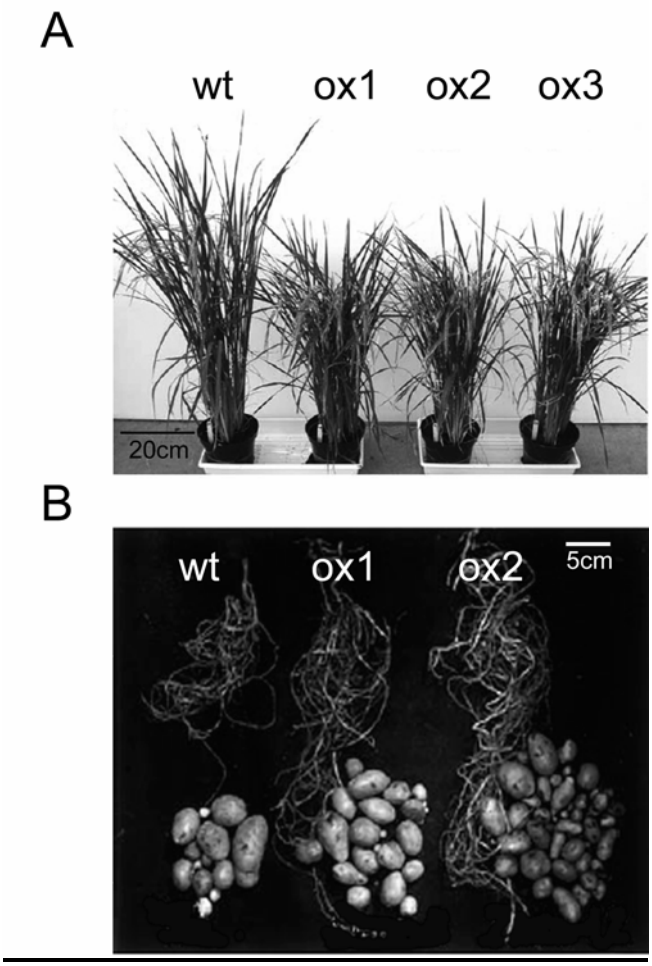

Figure 1. Dwarfing and yield increase using phytochrome overexpression.

A. Phenotype of greenhouse-grown rice plants overexpressing Arabidopsis phyA. The transgenic lines (ox1, ox2 and ox3) are all substantially shorter than wild type plants, and have shorter tiller internodes. The lines also showed a yield increase of 21%, 6% and 11% respectively. Adapted from Garg et al. (2005). Copyright Springer Publishing, 2005.

B. Increase in number and yield of tubers in potato plants overexpressing phytochrome B. The wild type (WT) and phyB-overexpressing transgenics (ox1 and ox2) were grown to harvest under greenhouse conditions. The ox2 line overexpresses phyB holoprotein at higher levels than ox1, consequently the tuber number increase is proportional to the level of phyB. Adapted from Thiele et al. (1999). Copyright American Society of Plant Biologists, 1999.

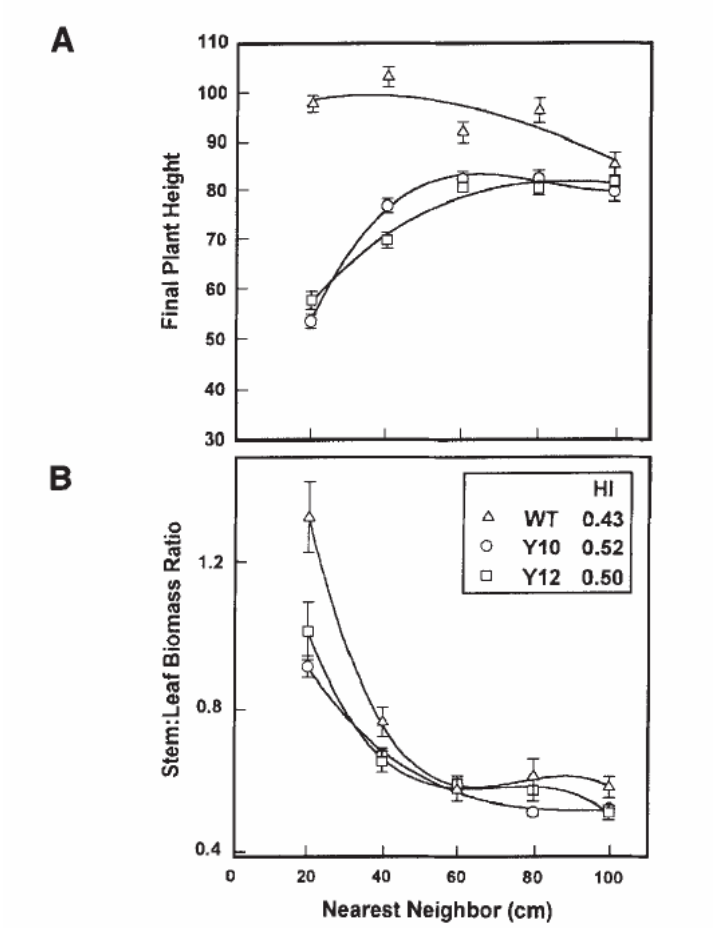

Figure 2. Proximity-conditional dwarfing of tobacco stands achieved with phyA overexpression

A) Plant heights at harvest, showing progressive dwarfing of the two transgenic lines (Y10 and Y12) as the nearest neighbor distance decreases. Wild type plants increase in height as the neighbor distance decreases due to the shade-avoidance syndrome.

B) Stem:Leaf biomass ratio and harvest index at the 20cm density for the same experiment. Note the dramatic increase in resource partitioning to the stem in wild type plants grown at high densities, the reduced impact of high-density growth on the transgenic lines, and the consequent improvement in harvest index for the transgenics.

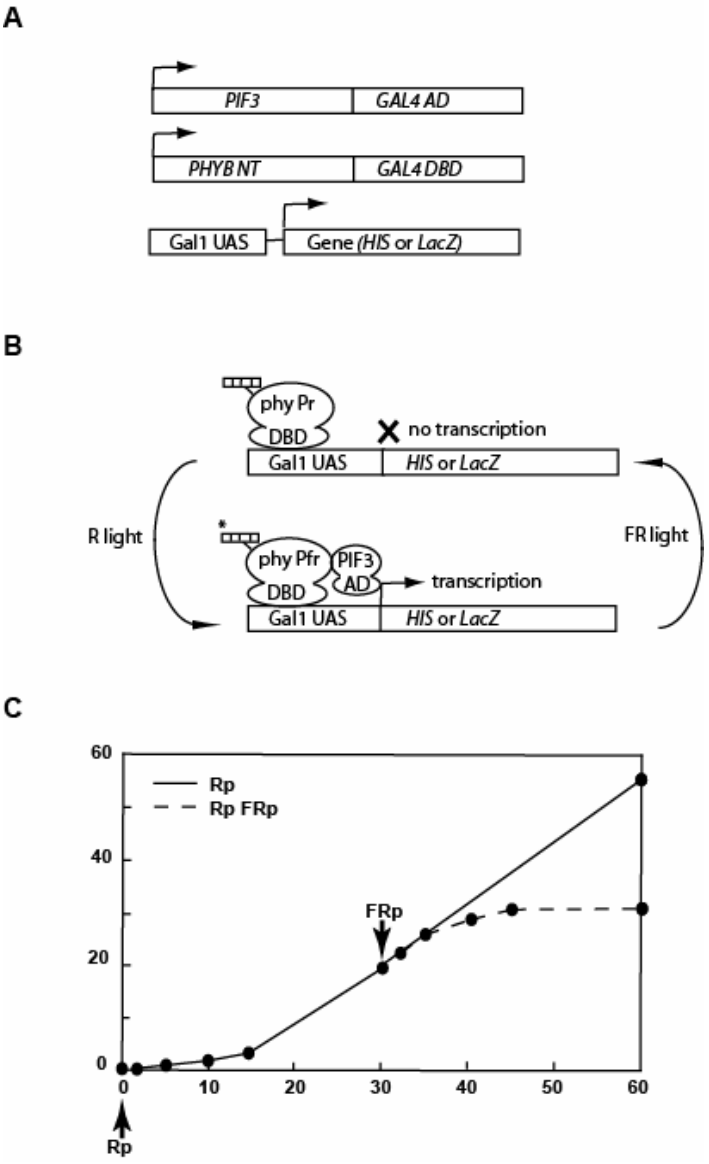

Figure 3. The use of light pulses, phytochrome and PIF3 to control gene expression in yeast.

A. Constructs are generated with *PIF3* and *PHYB* fused to the transcriptional activation and DNA-binding domain encoding portions of the yeast *GAL4* gene respectively. The gene to be controlled (in this case the *LacZ* or *HIS* reporter genes) is downstream from Gal1 UAS, the binding site of the GAL4 DNA binding domain.

B. *In vivo*, the phytochrome moiety is chromophorylated by the addition of exogenous phycocyanobilin (chromophore represented by four-box cartoon). The chromophorylated phytochrome moiety is anchored to the promoter of the reporter gene by the GAL4 DNA binding

domain. Unless a light pulse is provided, the phytochrome remains in the Pr form and no transcription occurs. However, once a pulse of red light is given, the phytochrome moiety converts to the Pfr form and can bind PIF3. The PIF3 – GAL4 activation domain fusion protein is recruited to the promoter, and transcription proceeds. However, this can be reversed at any point by a pulse of far-red light.

C. Response times of the system. A pulse of red light is given to the modified yeast cells, and detectable amounts of the LACZ enzyme begin to appear after 5-10 minutes. LACZ continues to accumulate linearly unless a pulse of FR light is given. With a lag time of 10 -15 minutes, accumulation of LACZ then ceases.

Adapted and redrawn from Shimizu-Sato et al., 2002 with permission.